BBA Report

BBA 61264

Cartilage collagen: Inability to serve as a substrate for collagenases active against skin and bone collagen

PAUL B. ROBERTSON and EDWARD J. MILLER

Institute of Dental Research, School of Dentistry, University of Alabama in Birmingham, Birmingham, Ala. 35294 (U.S.A.)

(Received September 11th, 1972)

SUMMARY

Human gingival and rabbit polymorphonuclear leukocyte collagenases previously shown to be active against native monomeric skin and bone collagen, with the chain composition $[\alpha 1(I)]_2\ \alpha 2$, do not cleave native monomeric cartilage collagen, with the chain composition $[\alpha 1(II)]_3$, as measured by a decrease in relative viscosity. Both types of collagen, however, are actively degraded by collagenase (EC 3.4.4.19) obtained from *Clostridium histolyticum*. The results strongly support the suggestion that collagen in cartilaginous structures is resorbed by a mechanism independent of that utilized in other tissues.

Biochemical studies on cartilage collagen have demonstrated that cartilaginous structures contain a genetically distinct $\alpha 1$ chain, designated $\alpha 1(II)$, in order to distinguish it from the $\alpha 1(I)$ and $\alpha 2$ chains common to the collagen in several other tissues such as bone, skin and tendon[1]. Further studies have shown that the $\alpha 1(II)$ chain is the predominant collagen chain in cartilaginous structures and that the chain composition of the majority of the collagen in these tissues may be characterized as $[\alpha 1(II)]_3$ (refs 2–7).

In considering possible physiological roles for the genetically distinct collagen in cartilage, it occurred to us that the process of endochondral bone formation would require that cartilage and bone collagens be degraded by mutually exclusive mechanisms due to the close proximity of resorbing cartilage and newly forming osteoid tissue.

It was predicted, therefore, that some specificity might exist with regard to the susceptibility of cartilage and bone collagen to collagenases active against undenatured collagen under physiological conditions. In the present investigation, we sought to study the

susceptibility of cartilage collagen, with the chain composition $[\alpha 1(II)]_3$, to particular collagenases known to be active against collagens of the chain composition $[\alpha 1(I)]_2 \alpha 2$.

Cartilage collagen was prepared from the sternal cartilages of 10-week-old White Leghorn chickens and articular cartilages of young calves. For chick cartilage the majority of the proteoglycan components were first removed from fresh cartilage slices by daily extraction at 4 °C with approx. 5 vol. of 1.0 M NaCl (pH 7.5, 0.05 M Tris) for 5 successive days. Following extraction, the slices were thoroughly rinsed at 4 °C in distilled water and lyophilized. For cartilage of bovine origin, the proteoglycan components were removed by extraction with 3.0 M guanidine·HCl at 25 °C (ref. 8). Following extraction, the slices were rinsed and lyophilized as above. Collagen was then solubilized by suspending 1 g of cartilage slices in 100 ml of 0.5 M acetic acid containing 100 mg of pepsin (PM, Worthington Biochemical Corp.) and incubating the suspension at 4 °C for 18 h. Cartilage collagen solubilized in this fashion was purified as previously described for acid-soluble bone collagen[9]. Characterization of the pepsin-solubilized cartilage collagen with respect to chain composition, molecular weight of the denatured chains, amino acid composition and cyanogen bromide peptides derived from the chains indicates that this collagen is comprised exclusively of molecules of the chain composition $[\alpha 1(II)]_3$ and differs from molecules of the same chain composition previously extracted directly from tissues of lathyritic animals[3,4] only with respect to the absence of short non-helical regions located at the amino- and carboxy-terminal regions of the molecule (Miller, E.J., unpublished results).

Acid-soluble collagen of the chain composition $[\alpha 1(I)]_2 \alpha 2$ was prepared and purified from chick bone[9] and rat skin[10] as previously described. In most experiments these collagens were preincubated with pepsin (EC 3.4.4.1) in order to mimic the conditions under which cartilage collagen was solubilized and thus assure that previous exposure to pepsin did not interfere with collagenase activity.

Gingival collagenase was recovered from 7-day-pooled culture media as previously described[11]. Polymorphonuclear leukocyte collagenase was obtained directly from granules of rabbit peritoneal exudate leukocytes by homogenisation procedures recently reported[12]. Collagenase (EC 3.4.4.19) from *Clostridium histolyticum* (Type III, Fraction A, C-0255) was obtained from Sigma Chemical Company,

All viscometry was performed at 25 °C with collagen dissolved as native monomeric molecules at a concentration of 1 mg/ml in a solution containing 0.005 M $CaCl_2$, 0.2 M NaCl, 0.03 M Tris, pH 7.5. The collagen solutions (5 ml) were brought to 25 °C and incubation was started by addition of 1 ml of test solutions which consisted of collagenases in the same solvent, trypsin (EC 3.4.4.4) (0.5 mg/ml; TRTPCK, Worthington Biochemical Corp.) in the same solvent and the solvent alone as control.

As depicted in Fig. 1 for rat skin and chick cartilage collagens, the collagen preparations were highly resistant to general protease activity and reaction mixtures containing trypsin (1 mg trypsin per 10 mg collagen) did not exceed a 5% decrease in relative viscosity. In contrast, the relative viscosity of rat skin collagen incubated with gingival or polymorphonuclear leukocyte collagenases was reduced by approximately 30% over the time period examined. Identical results were achieved when chick bone collagen was substituted for rat

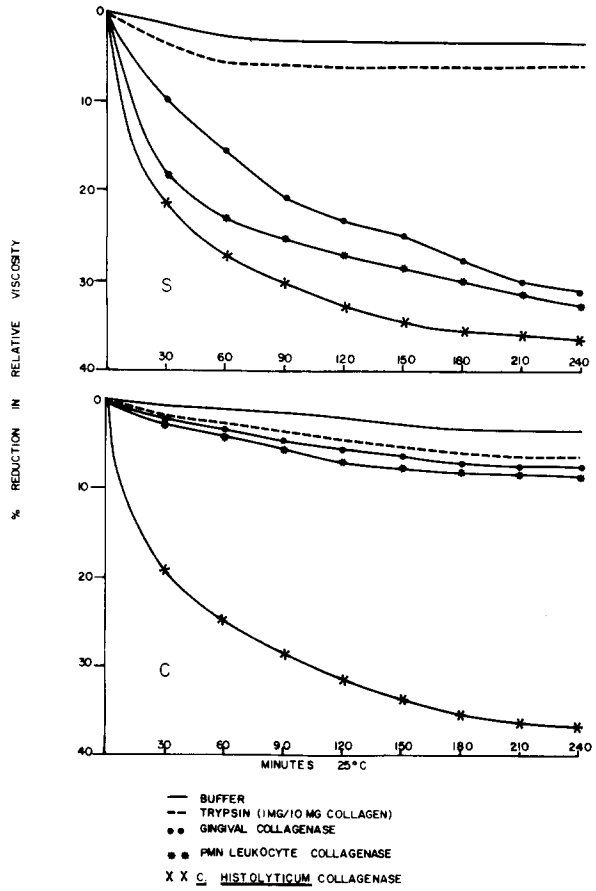

Fig. 1. Percent reduction in relative viscosity of skin collagen (S) and cartilage collagen (C) incubated under the conditions indicated. The percent reduction of relative viscosity of cartilage collagen (C) subjected to gingival and polymorphonuclear (PMN) leukocyte collagenases was equal to values obtained with trypsin after 240 min, although some separation is shown in Fig. 1 for the purpose of clarity.

skin collagen in the assay and preincubation of either collagen preparation with pepsin did not alter the results. As shown in the lower portion of the figure, no significant reduction in the relative viscosity of either bovine articular or chick sternal cartilage collagen was apparent when incubated with either gingival or polymorphonuclear leukocyte collagenase. As expected, in view of the much wider specificity of bacterial collagenase, the collagen preparations utilized in this study exhibited no differential reactivity with respect to the latter enzyme and the decrease in relative viscosity observed was virtually identical for all preparations when incubated with bacterial collagenase.

Although the existence of a vertebrate collagenase that specifically cleaves native cartilage collagen has not as yet been demonstrated, the results presented here suggest that collagenases produced by polymorphonuclear leukocytes and cells of chronic inflammatory

tissue do not significantly contribute to the initial cleavage of native cartilage collagen.

Gingival collagenase and polymorphonuclear leukocyte collagenase differ with respect to availability within their respective tissues, method of extraction, and inhibition by serum proteins. Nevertheless, both enzymes appear to cleave native skin and bone collagen at the same site, resulting in two fragments approximately three-quarter and one-quarter the length of the original molecule[13]. Neither collagenase exhibits activity when cartilage collagen is used as a substrate which may be a function of the different primary structure of the $\alpha 1(II)$ chains comprising the cartilage collagen molecule or alternatively may reflect the absence of an $\alpha 2$ chain characteristic of the collagen susceptible to these enzymes. Information with respect to the amino acid sequences at the locus of collagenase cleavage in molecules of the chain composition $[\alpha 1(I)]_2 \alpha 2$ will be of considerable importance in evaluating these alternatives.

This work was supported by U.S. Public Health Service Grant DE-02670.

REFERENCES

1 Miller, E.J. and Matukas, V.J. (1969) *Proc. Natl. Acad. Sci. U.S.* 64, 1264–1268
2 Trelstad, R.L., Kang, A.H., Igarashi, S. and Gross, J. (1970) *Biochemistry*, 9, 4993–4998
3 Miller, E.J. (1971) *Biochemistry* 10, 1652–1659
4 Miller, E.J., (1971) *Biochemistry* 10, 3030–3035
5 Miller, E.J., Epstein, Jr. E.H. and Piez, K.A. (1971) *Biochem. Biophys. Res. Commun.*, 42, 1024–1029
6 Strawich, E. and Nimni, M.E. (1971) *Biochemistry* 10, 3905–3911
7 Toole, B.P., Kang, A.H., Trelstad, R.L. and Gross, J. (1972) *Biochem. J.* 127, 715–720
8 Sagdera, S.W. and Hascall, V.C. (1969) *J. Biol. Chem.*, 244, 77–87
9 Miller, E.J., Martin, G.R., Piez, K.A. and Powers, M.J. (1967) *J. Biol. Chem.* 242, 5481–5489
10 Bornstein, P. and Piez, K.A. (1966) *Biochemistry* 5, 3460–3473
11 Fullmer, H.M. and Lazarus, G.S. (1969) *J. Histochem. Cytochem.* 17, 793–798
12 Robertson, P.B., Ryel, R.B., Taylor, R.E., Shyu, K.W. and Fullmer, H.M. (1972) *Science* 177, 64–65
13 Fullmer, H.M. (1970) in *Metabolic Conjugation and Metabolic Hydrolysis* (Fishman, W.H., ed.), Vol. 2, pp. 301–343, Academic Press, New York and London